PURIFICATION AND CHARACTERIZATION OF SERINE PROTEINASES FROM *BOTHROPS* SNAKE VENOMS

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Snake venoms are important sources of substances with a variety of pharmacological activities. Snake venom serine proteases (SVSPs) have important effects on the hemostatic system that influence the hemodynamic properties of blood. The aim of this work was to isolate the TLEs from five *Bothrops* snake venoms (*Bothrops alternatus*, *B. jararaca, B. jararacussu, B. moojeni* and *B. pauloensis*). The venoms were fractionated by reversed-phase liquid chromatography (process under patent). The enzymes were characterized using SDS-PAGE and in-gel protein digestion coupled to LC-MS/MS and Mascot protein identification, and their coagulant activity was assessed. TLEs from *B. moojeni* and *B alternatus* were isolated in two chromatographic steps with good protein yield, while those of *B. jararaca, B. jararacussu* and *B. pauloensis* required additional steps. All TLEs had molecular masses of 30-40 kDa and their partial sequences shared high identity with venom plasminogen activator, platelet-aggregating enzyme, SVSPs and batroxobin from *Bothrops* venoms. The rapid purification and good yield of TLEs from *B. moojeni* and *B. alternatus* should provide new acknowledgements about these enzymes.

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References

ALEXANDER, G.; GROTHUSEN, J.; ZEPEDA, H.; SCHWARTZMAN, R.J. Giroxin, a toxin from the venom of Crotalus durissus terrificus is a trombin-like enzyme. Toxicon, v. 26, p. 953-960, 1988.

BARROS, L.C.; FERREIRA, J.R.R.S.; BARRAVIERA, S.R.S.C.; STOLF, S.H.O.; THOMAZINI-SANTOS, I.A.; MENDES-GIANNINI, M.J.S.; TOSCANO, E.; BARRAVIERA, B. A new fibrin sealant from Crotalus durissus terrificus venom: applications in medicine. Journal of Toxicology and Environmental Health, Part B, v. 12, p. 1-19, 2009.

BERNARDES, C.P.; SANTOS-FILHO, N.A.; COSTA, T.R.; GOMES, M.S.R.; TORRES, F.S.; COSTA, J.; BORGES, M.H.; RICHARDSON, M.; SANTOS, D.M.; PIMENTA, A.M.C.; BRANDEBURGO, M.I. H.; SOARES, A.M.; OLIVEIRA, F. Isolation and structural characterization of a new fibrin(ogen)olytic metalloproteinase from Bothrops moojeni snake venom. Toxicon, v. 51, p. 574–584, 2008.

COSTA, F.L.S.; RODRIGUES, R.S.; IZIDORO, L.F.M.; MENALDO, D.L.; HAMAGUCHI, A.; HOMSI-BRANDEBURGO, M.I.; FULY, A.L.; SOARES, S.G.; SELISTRE-DE-ARAÚJO, H.S.; BARRAVIERA, B.; SOARES, A. M., RODRIGUES, V.M. Biochemical and functional properties of a thrombin-like enzyme isolated from Bothrops pauloensis snake venom. Toxicon, v. 54, p. 725-735, 2009.

COSTA, J.O.; FONSECA, K.C.; MAMEDE, C.C.N.; BELETTI, M.E.; SANTOS-FILHO, N.A.; SOARES, A.M.; ARANTES, E.C.; HIRAYAMA, S.N.S.; SELISTRE-DE-ARAÚJO, H.S.; FONSECA, F.; HENRIQUE-SILVA, F.; PENHA-SILVA, N.; OLIVEIRA, F. Bhalternin: Functional and structural characterization of a new thrombin-like enzyme from Bothrops alternatus snake venom. Toxicon, v. 55, p. 1365–1377, 2010.

Isolation of TLE serine proteases from five *Bothrops* species venoms

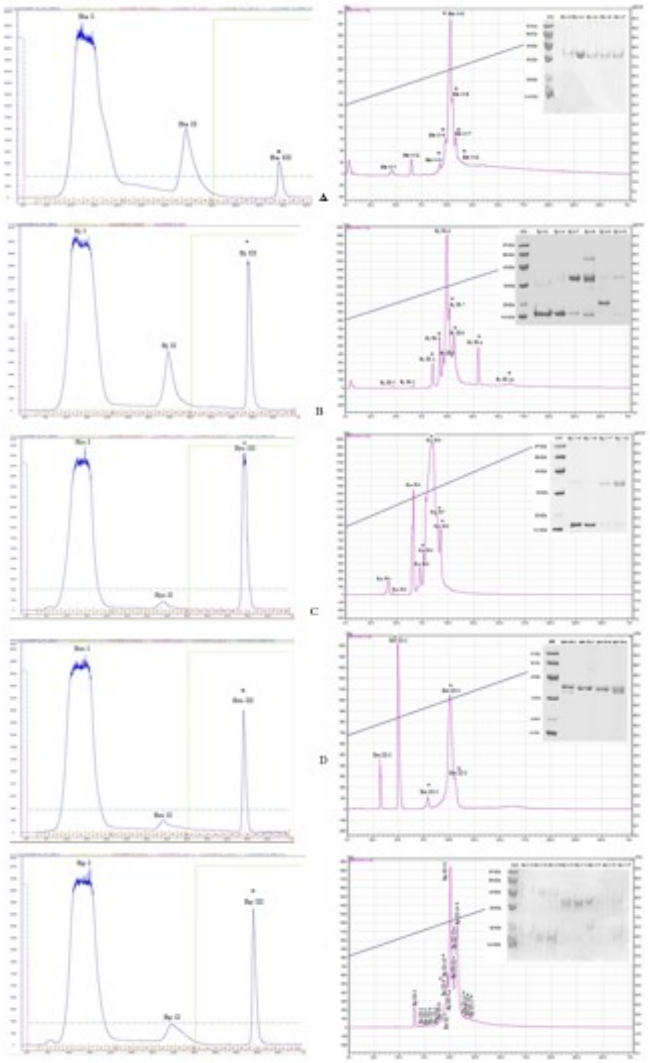
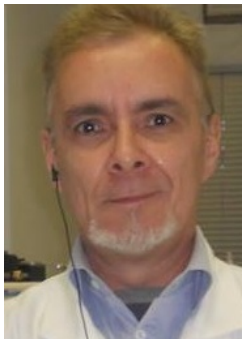


Figure 1) 500 mg of crude venom of each *Bothrops* snake were applied to a Benzamidine-Seharose 6B affinity column. The first column figures represents the chromatograms obtained in this experimental phase, where the serine proteases were located in peak III. Subsequently, the fraction III was subjected to RP-HPLC (second column) in a Shimadzu C18 column, revealing new fractions. The fractions marked with ***** showed coagulant activity. **Insert:** Electrophoretic profiles on SDS-PAGE (13%) under denaturing and reducing conditions. **A**- *B. alternatus*; **B**- *B. jararaca*; **C**- *B. jararacussu*; **D**- *B. moojeni*; **E**- *B. pauloensis*.



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